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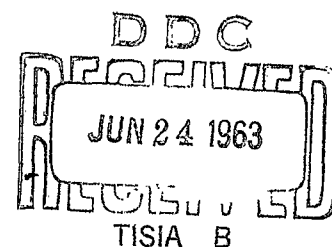
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EFFECTS OF COLD ACCLIMATIZATION ON LIVER
DI- AND TRIPHOSPHOPYRIDINE NUCLEOTIDE

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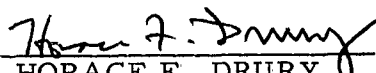
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ABSTRACT

The levels of oxidized and reduced di- and triphosphopyridine nucleotide were measured in liver tissue from rats that had been exposed to cold ($4^{\circ} \pm 1^{\circ} \text{C}$) for one month. These animals exhibited about 65% more TPNH and total triphosphopyridine nucleotide than control animals maintained at an ambient temperature of $25^{\circ} \pm 1^{\circ} \text{C}$. The significance of these alterations to the efficiency of oxidative phosphorylation is discussed.

PUBLICATION REVIEW



HORACE F. DRURY
Director of Research

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SECTION 1. INTRODUCTION

Recent in vitro investigations have indicated that the improved thermogenic capacity of the cold-acclimatized animal is due, at least in part, to alterations in the pattern and magnitude of electron transport. Grossly, these alterations are reflected by an elevated activity of the tricarboxylic acid cycle (Hannon, 1958) and a reduced efficiency of oxidative phosphorylation (Hannon, 1959; Smith and Fairhurst, 1958; Panagos et al, 1958). Within the electron transport system an increased activity or concentration of succinic and malic dehydrogenase (Hannon, 1960), coenzyme Q (Beyer et al, 1962), cytochrome c (Klain, 1961) and cytochrome oxidase (Hannon, 1960) has been observed in various tissue preparations from chronically-exposed rats. In addition, the liver of the cold-acclimatized hamster was reported (Reynafarje and Chaffee, 1960) to have an increased activity of DPNH- and TPNH-³ cytochrome c reductase. However, alterations in these latter two enzymes, as well as lactic dehydrogenase and TPNH-DPN transhydrogenase, were not observed in liver tissue from acclimatized rats (Smith and Fairhurst, 1958; Hannon, 1960). The present report is concerned with the effect of cold acclimatization on two additional components of the electron transport system, namely, di- and triphosphopyridine nucleotide.

The abbreviations TPN, TPNH, and DPN, DPNH used herein refer to oxidized and reduced triphosphopyridine and diphosphopyridine nucleotides, respectively.

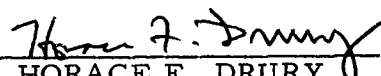
SECTION 2. METHODS

Adult, male, Sprague-Dawley rats, weighing between 300 and 350 g at the beginning of the study, were randomly segregated into two groups of seven animals each. They were placed in individual wire cages and maintained at all times on an ad libitum diet of Purina Laboratory Checkers and water. One group was subjected to cold ($4^{\circ} \pm 1^{\circ} \text{C}$) exposure for a period of one month to induce cold acclimatization. The other group was maintained in the animal colony ($25^{\circ} \pm 1^{\circ} \text{C}$) as controls.

ABSTRACT

The levels of oxidized and reduced di- and triphosphopyridine nucleotide were measured in liver tissue from rats that had been exposed to cold ($4^{\circ} \pm 1^{\circ}$ C) for one month. These animals exhibited about 65% more TPNH and total triphosphopyridine nucleotide than control animals maintained at an ambient temperature of $25^{\circ} \pm 1^{\circ}$ C. The significance of these alterations to the efficiency of oxidative phosphorylation is discussed.

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At the time of tissue analysis, control and cold-acclimatized rats were alternately brought into the laboratory where they were killed by a blow on the head. The liver of each animal was rapidly excised, wiped free of excess blood, and frozen in an ethanol-dry ice mixture (about -70°C). While still frozen, the tissue was mechanically crushed, and weighed samples were transferred to glass and Teflon homogenizers which had been prefilled with either cold (0°C) 2% perchloric acid (DPN and TPN determinations) or with hot (100°C) 0.1 M Na_2CO_3 (DPNH and TPNH determinations). Following homogenization for two minutes the samples were chilled, subjected to centrifugation for the removal of denatured protein, and neutralized to pH 7.0.

The pyridine nucleotide concentrations in the tissue extracts were determined spectrophotometrically according to the procedure of Ciotti and Kaplan (1957). Briefly, DPN and TPN concentrations were measured with yeast alcohol dehydrogenase and isocitric dehydrogenase methods, respectively, while the DPNH and TPNH concentrations were measured with the yeast alcohol dehydrogenase and glutathione reductase methods, respectively. With the exception of the glutathione reductase, all of the aforementioned reagents were commercial preparations (Sigma). Glutathione reductase was prepared in the laboratory from dried peas according to the method outlined by Ciotti and Kaplan (1957).

The concentrations of the various nucleotides were calculated, and are reported here, in terms of the original tissue wet weight, that is, as $\mu\text{ moles/g}$ of liver. They were also calculated in terms of tissue nitrogen, but since the protein concentration of the liver was unaffected by one-month cold exposure, essentially the same results were obtained. Fisher's t test was used to assess the significance of differences between means.

SECTION 3. RESULTS

Chronic cold exposure induced a marked (about 65%) increase in the concentration of liver TPNH, but had no apparent effect on the concentrations of TPN, DPN or DPNH. This change in TPNH was directly responsible for a significant ($P < 0.01$) rise in the total triphosphopyridine nucleotide (i. e., $\text{TPN} + \text{TPNH}$). Thus, the overall effect of cold acclimatization was a decrease in the molecular ratio of total diphosphopyridine nucleotide to total triphosphopyridine nucleotide from about 3 to 1 to about 2 to 1.

As observed in an earlier study (Hannon and Vaughan, 1961), the liver to body weight ratio was not significantly increased by cold exposure for a period of one month. Consequently, the total amounts of pyridine nucleotide

available for the support of liver function in the animal as a whole were not influenced by a change in relative liver size. Such an influence, however, might obtain in animals subjected to cold exposure for several months since such animals have been shown (Hannon and Vaughan, 1961) to exhibit a highly significant increase in the liver to body weight ratio.

TABLE I

Effect of Cold Acclimatization on Di- and
Triphosphopyridine Levels in the Liver

Component	Control Rats Mean* S. E.	Cold Acclim. Rats Mean S. E.	Δ	P
DPN	0.476 \pm 0.042	0.455 \pm 0.018	0.021	n. s.
DPNH	0.013 \pm 0.003	0.013 \pm 0.003	0	n. s.
DPN+DPNH	0.489 \pm 0.045	0.468 \pm 0.022	0.021	n. s.
TPN	0.007 \pm 0.001	0.008 \pm 0.001	0.001	n. s.
TPNH	0.145 \pm 0.026	0.239 \pm 0.029	0.094	<0.01
TPN+TPNH	0.152 \pm 0.027	0.247 \pm 0.031	0.095	<0.01
<u>DPN+DPNH</u> <u>TPN+TPNH</u>	3.15	1.89		

* Expressed as μ moles/g liver
n. s. not significant

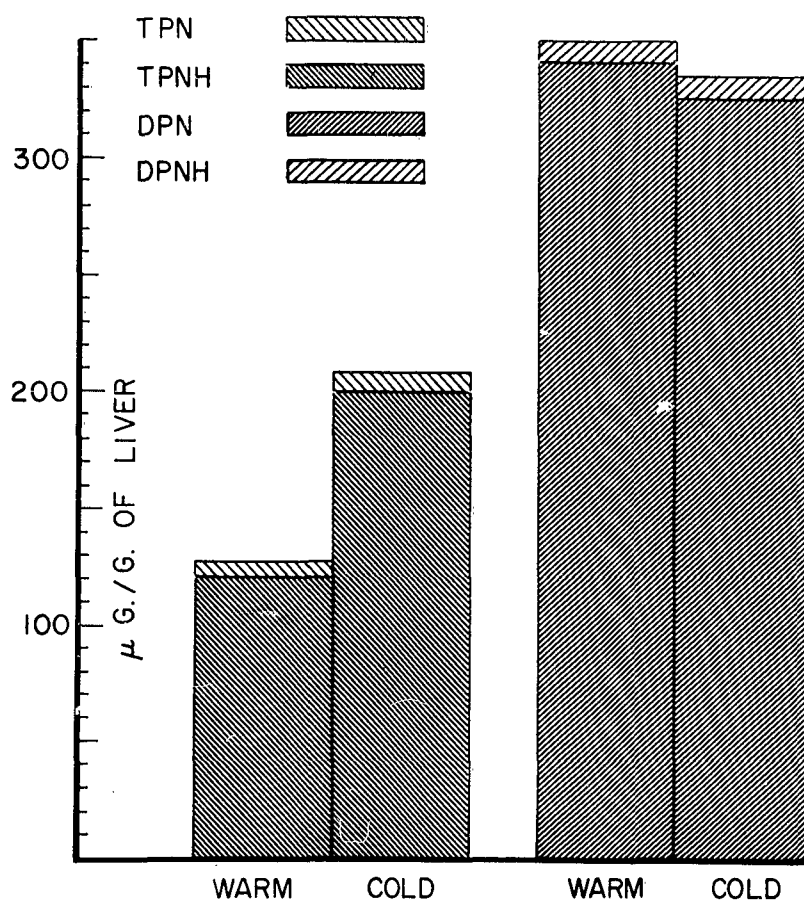


FIGURE 1.

SECTION 4. DISCUSSION

Although it is generally agreed that metabolic cold acclimatization includes a reduction in the efficiency of oxidative phosphorylation (Hannon, 1959; Smith and Fairhurst, 1958; Panagos et al, 1958), there are conflicting data and opinions regarding the mechanisms responsible for this phenomenon. Potter (1958) was perhaps the first to suggest that it might be due to shifts in the pattern of electron transport. Specifically, he proposed that cold exposure might lead to a relative increase in the activity of the poorly phosphorylating (Kaplan et al, 1956) TPNH-cytochrome c reductase pathway. Early experiments, however, gave little support to Potter's proposal. Thus, studies (Smith and Fairhurst, 1958; Hannon, 1960) of mitochondrial and homogenate preparations failed to show any cold-induced alterations in the

activities of DPNH- and TPNH cytochrome c reductase or of TPNH-DPN transhydrogenase. Such alterations, especially in the case of TPNH cytochrome c reductase and transhydrogenase, might be expected if an alternate pathway of electron transport were being emphasized.

In somewhat later studies of liver mitochondrial preparations from cold acclimatized rats, Smith (1959) showed that the reduction in the P/O ratio was probably due to the presence of certain, poorly defined, extramitochondrial factors. Subsequently, Reynafarje and Chaffee (1960) reported an increase in the DPNH and TPNH cytochrome c reductase activities of microsomal but not mitochondrial preparations of liver tissue from cold-acclimatized hamsters. Since the increase was relatively greater in the case of the TPNH- cytochrome c reductase, these experiments would seem to be in agreement with Potter's proposed mechanism for lowering the P/O ratio. It should be noted, however, that hamsters are a hibernating species, and the foregoing changes may be related to preparations for the hibernating state.

Beyer et al (1962) have quite recently reported exceptionally high concentrations of coenzyme Q in various tissues from cold-acclimatized rats and suggest that this coenzyme may serve as a bypass to the phosphorylation that normally occurs prior to cytochrome b. If future experiments verify this suggested function of coenzyme Q, a second mechanism for lowering the P/O ratio would be available.

In the present experiments the high TPNH and total triphosphopyridine nucleotide levels would provide added evidence that cold acclimatization leads to an increased emphasis of a poorly-phosphorylating, probably extramitochondrial, pathway of electron transport. These experiments, however, give no direct information on the mechanisms that might be responsible for either of these changes. The elevated concentration of TPNH could result from an increase in the rate of isocitrate oxidation (Hannon, 1958), but it is not likely to be the result of an increase in the activity of the hexosemonophosphate shunt. With respect to the latter, available evidence shows the activities of the TPNH-generating shunt enzymes to be either unaffected (Smith and Fairhurst, 1958; Vaughan et al, 1961) or reduced (Hannon and Vaughan, 1960) following prolonged cold exposure.

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Arctic Aeromedical Laboratory, United States Air Force (AFSC), APO 731, Seattle, Wash. Rpt. AAL-TDR-62-40. EFFECTS OF COLD ACCLIMATIZATION ON LIVER DI- AND TRIPHOSPHOPYRIDINE NUCLEOTIDE. January 1963. 7 p. incl. table, illus., 15 ref.	<ol style="list-style-type: none"> 1. Nucleotides 2. Liver 3. Rats 4. Exposure 5. Enzymes <p>I. Project 8237-02 II. Hannon, J. P. and A. Rosenthal III. Available from OTS IV. In ASTIA collection</p> <p>Unclassified Report</p> <p>The levels of oxidized and reduced di- and triphosphopyridine nucleotide were measured in rat liver tissue from rats that had been exposed to cold ($4^{\circ} \pm 1^{\circ} \text{C}$) for one month. These animals exhibited about 65% more TPNH and total triphosphopyridine nucleotide than control animals maintained at an ambient temperature of $25^{\circ} \pm 1^{\circ} \text{C}$. The significance of these alterations to the efficiency of oxidative phosphorylation is discussed.</p>
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